APA520Hu01 100μg
Active Protein Kinase R (PKR)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Ser224~Ile502
Tags: N-terminal His-tag
Purity: >80%
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 8.9
Predicted Molecular Mass: 35.8kDa
Accurate Molecular Mass: 32kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

SSSLLMN GLRNNQRKAK RSLAPRFDLP
DMKETKYTVD KRFGMDFKEI ELIGSGGFGQ VFKAKHRIDG KTYVIKRVKY
NNEKAEREVK ALAKLDHVNI VHYNGCWDGF DYDPETSDDS LESSDYDPEN
SKNSSRSKTK CLFIQMEFCD KGTLEQWIEK RRGEKLDKVL ALELFEQITK
GVDYIHSKKL IHRDLKPSNI FLVDTKQVKI GDFGLVTSLK NDGKRTRSKG
TLRYMSPEQI SSQDYGKEVD LYALGLILAE LLHVCDTAFE TSKFFTDLRD
GI

[ACTIVITY]

Protein Kinase R (PKR) is activated by double-stranded RNA (dsRNA), the synthesis of which is caused virally. PKR can also be activated by the protein PACT or by heparin. It plays a key role in the innate immune response to viral infection and is also involved in the regulation of signal transduction, apoptosis, cell proliferation and differentiation. Besides, Cyclin Dependent Kinase 1 (CDK1) has been identified as an interactor of PKR, thus a binding ELISA assay was conducted to detect the interaction of recombinant human PKR and recombinant

human CDK1. Briefly, PKR were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to CDK1-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-PKR pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of of PKR and CDK1 was shown in Figure 1, and this effect was in a dose dependent manner.

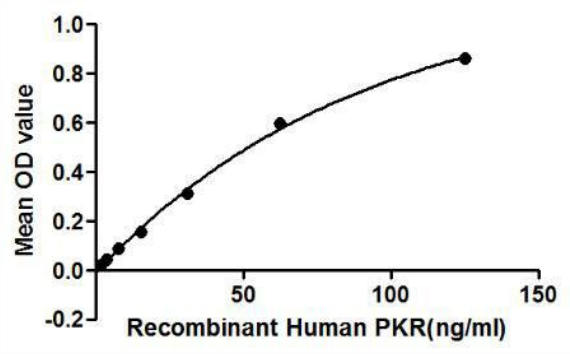

Figure 1. The binding activity of PKR with CDK1.

[IDENTIFICATION]

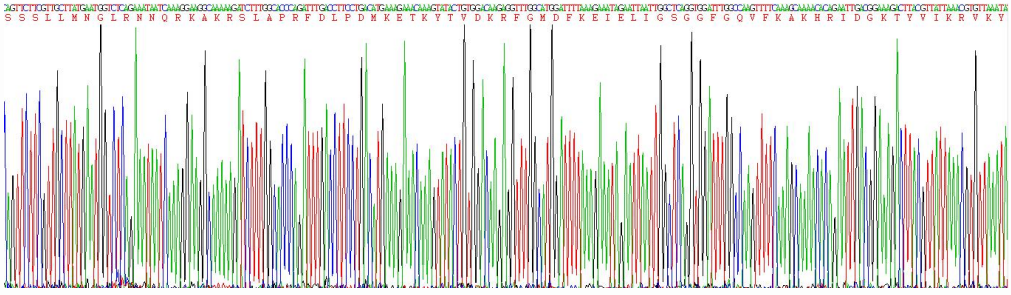

Figure 2. Gene Sequencing (extract)

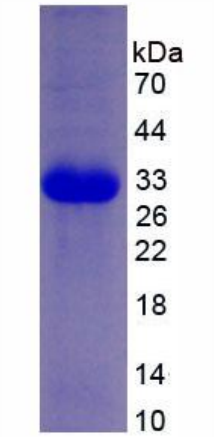

Figure 3. SDS-PAGE

Sample: Active recombinant PKR, Human

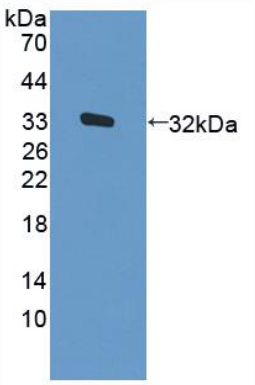

Figure 4. Western Blot

Sample: Recombinant PKR, Human;

Antibody: Rabbit Anti-Human PKR Ab (PAA520Hu01)